

## SPECIFICATION AMENDMENT

The present invention will now be more specifically described with the following examples; ~~and the accompanying figures.~~

~~Figure 1 shows a mature antisense embryo.~~

~~Figure 2 shows an antisense seedling.~~

~~Figure 3 shows the cloning scheme for obtaining the ASK dzetha antisense construct.~~

SEQ ID No. 1 to 5 represent oligonucleotide primers used to generate ASK specific DNA fragments, and for their detection in transformed plants.

## SPECIFICATION AMENDMENT

PCR reactions were carried out using 1 ng of ASK cDNA as a template and the following reaction conditions: initial denaturation at 94°C for 2 min., followed by 35 cycles of 94°C/30s, 45°C/30s and 72°C/1 min. PCR products were cloned as *Xba*I-*Cla*I fragments into the pBlueScript (Stratagene) vector (~~Fig. 3~~) and sequenced on both strands to check for polymerase induced errors.

## SPECIFICATION AMENDMENT

These fragments were cloned in antisense orientation under the strong constitutive CaMV 35S promoter, in a modified version of the *Agrobacterium tumefaciens* pEC<sub>2</sub> plasmid (INRA, Versailles, France ~~plasmid map, Fig. 3~~). ~~Fig. 3 shows the~~ The cloning scheme is indicating that the ASK-dzetha 5' region defined above is cloned in antisense orientation to the 35S CaMV promoter and is functionally linked to the 35S CaMV 3' transcription termination region (construct: ASK  $\alpha$  AS). The expression cassette obtained is cloned together with the bar gene between the left and right border sequence of *Agrobacterium tumefaciens*. At least 18 plants were obtained for each of the ASK antisense constructs. Transformation, cultivation and regeneration were carried out using standard protocols. Transformed plants were left to self-pollinate and the progeny was tested for the presence of the construct insertion by PCR using primers in the ASK genes in combination with the TAG17 primer on the pEC<sub>2</sub> T-DNA (5'-GAGCCGCAG GAACCGCAGGAGTGCA-3', SEQ ID No. 5). The amount of native ASK (about 1,6 kbp) and antisense (about 0,3 kbp) transcript levels was accessed by Northern blot experiments, using ASK gene-specific probes under the conditions described in Dornelas et al., 1999.

## SPECIFICATION AMENDMENT

Both in the wild-type as in the ASK $\zeta$  antisense embryos, at the late globular to heart stage protodermal divisions increased in frequency at the site of the future cotyledons. These cell divisions resulted in a triangular shaped embryo. The cotyledon initials which were formed at the apical region of the ASK $\zeta$  antisense embryo were supernumerary in most cases (70% of the embryos analysed, n>100). Thus, when cells that will form the cotyledons are recruited at the late globular stage of ASK $\zeta$  antisense embryos, as much as twice the amount of cells were available. Consequently, up to six cotyledons were detected in mature ASK $\zeta$  antisense embryos (Fig. 1). At the torpedo stage, the supernumerary cotyledons were visible in cleared seeds. With further elongation of the cotyledons and the bending of the embryo, the seeds of the ASK $\zeta$  antisense plants showed a roundish shape when compared to the wild-type, due to the accommodation of the supernumerary cotyledons.

## SPECIFICATION AMENDMENT

After germination the ASK $\zeta$  antisense seedlings displayed a normal shape, except that they showed an increased number of cotyledons (polycotyly) (~~Fig. 2~~). Ninety percent of the seedlings presenting polycotyly showed 3 cotyledons, while ten percent showed 4-6 cotyledons. In this latter case, cotyledons were reduced in size. The relative position of the first leaves, which alternate with the insertion of cotyledons, was maintained in the ASK $\zeta$  antisense plants.